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MINIREVIEW

Prevalence and Significance of Plasmid Maintenance Functions in the Virulence Plasmids of Pathogenic Bacteria▽

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Virulence functions of pathogenic bacteria are often encoded on large extrachromosomal plasmids. These plasmids are maintained at low copy number to reduce the metabolic burden on their host. Low-copy-number plasmids risk loss during cell division. This is countered by plasmid-encoded systems that ensure that each cell receives at least one plasmid copy. Plasmid replication and recombination can produce plasmid multimers that hinder plasmid segregation. These are removed by multimer resolution systems. Equitable distribution of the resulting monomers to daughter cells is ensured by plasmid partition systems that actively segregate plasmid copies to daughter cells in a process akin to mitosis in higher organisms. Any plasmid-free cells that still arise due to occasional failures of replication, multimer resolution, or partition are eliminated by plasmid-encoded postsegregational killing systems. Here we argue that all of these three systems are essential for the stable maintenance of large low-copy-number plasmids. Thus, they should be found on all large virulence plasmids. Where available, well-annotated sequences of virulence plasmids confirm this. Indeed, virulence plasmids often appear to contain more than one example conforming to each of the three system classes. Since these systems are essential for virulence, they can be regarded as ubiquitous virulence factors. As such, they should be informative in the search for new antibacterial agents and drug targets.

Pathogenic bacteria differ from their harmless relatives in having genes for virulence factors that facilitate host invasion and infection. These factors either are encoded by the host chromosome or are carried on mobile genetic elements. The latter include transposons, viral prophages, and plasmids. Mobile elements provide an extensive library of potentially useful functions that can be readily adopted for the rapid adaptation of the bacterium to its environment. As these elements are acquired from other organisms in the biosphere, the bacterium has the potential to express a wide array of virulence-associated functions without the burden of carrying all the genetic information involved. Only those virulence factors required for the current adaptation need be carried. Since mobile elements are able to reassort the functions they carry by interaction with other elements in the biosphere, the variety and assortment of functions that can be acquired by transfer of an element are virtually unlimited.

Virulence plasmids are usually transmitted between hosts by conjugation. They often carry many virulence genes in addition to functions for plasmid transmission and maintenance and are therefore large (Table 1). Large plasmids are always present in low copy numbers in their bacterial host. Otherwise, the metabolic burden of maintaining and duplicating their genomes would be excessive. This compromises their chance of being faithfully transmitted to daughter cells during cell division, because random distribution of plasmid copies cannot ensure inheritance at low copy number (51). Although acquisition and loss of plasmids constitute an important asset for evolution, the plasmids must be inherited stably on a shorter time scale in order to minimize random losses at cell division. This is especially important in the reservoir of infective cells outside the infected host, where there is no selection for the virulence factors that the plasmid carries.

Three principal classes of plasmid maintenance function have been described. Multimer resolution systems resolve plasmid multimers caused by replication and recombination among sister plasmids, thereby maximizing the number of plasmid copies available for segregation (3). Postsegregational killing systems kill cells that lose the plasmid, thus ensuring the continued presence of the plasmid in the bacterial population (73). Finally, partition systems actively segregate sister plasmids to daughter cells by a process that is analogous to the mitotic segregation of chromosomes (18). Since these three types of system combine to ensure high levels of segregation fidelity (51), we argue that all three types of system are likely to be present on all large virulence plasmids. These systems should be critical for pathogenesis and may be informative for the development of novel antibacterial agents.

There is no uniform nomenclature for plasmid replication or maintenance systems. Sometimes terms that are generally used for one type of system have been used for another type in the case of a specific plasmid. For example, the term *par*, which is generally used for partition systems, refers to a gene for a multimer resolution recombinase in the case of plasmid RP4 (17) and to a postsegregational killing system component gene in pAD1 (72).

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TABLE 1. Examples of plasmid maintenance systems in virulence plasmids[a]

Organism and plasmid	Size (bp)	Partition system	*par* type(s)	PSK	Multimer resolution system
Enteropathogenic *E. coli* (EPEC) pB171	68,817	ParA/ParB,[b] ParR/ParM[b]	Ib, II	CcdA/CcdB[c]	RsvA,[e] RsvB[e]
E. coli pO113	165,548	StbA/StbB	II	PndA/PndC, CcdA/CcdB[c]	ResD[e]
Salmonella enterica serovar Typhimurium pSLT1	93,939	ParA/ParB	Ia	CcdA/CcdB[c]	RsdB[e]
Salmonella enterica pOU1113	80,156	ParA/ParB	Ia	CcdA/CcdB[c]	RsdB[e]
Salmonella Dublin pOU1115	74,589	ParF/ParG	Ib	CcdA/CcdB,[c] StbE/StbD	RsdB[e]
Shigella flexneri pWR501	221,851	ParA/ParB, StbA/StbB	Ia, II	CcdA/CcdB,[c] MvpA/MvpT,[d] RelB	TnpR, *cer*[e]

[a] All information compiled from NCBI site for bacterial plasmid sequences.
[b] Compiled from reference 19.
[c] Homologous to the F plasmid PSK system, CcdA/CcdB (10).
[d] Homologous to the pMYS6000 PSK system, MvpA/MvpT (58).
[e] Tyrosine recombinase system components (62).

RESOLUTION OF PLASMID MULTIMERS

Homologous recombination between sister plasmids during or after replication can readily give rise to plasmid dimers or higher multimers. This decreases the number of plasmid copies available for segregation to daughter cells. Since many large plasmids are present as only two or three copies per cell, such events would lead to frequent plasmid loss (3, 51). In addition, the type of replication control used by many plasmids makes them vulnerable to a cumulative effect of dimer formation termed "dimer catastrophe" (63), further decreasing plasmid stability. For accurate segregation, it is important that multimers are resolved to monomers prior to cell division. This is accomplished by enzyme-mediated, site-specific recombination systems. Large plasmids encode their own recombinase systems consisting of genes for a specific recombinase and a recombination site at which they act (Table 1). Dimers contain two such sites that are cut, exchanged, and rejoined by the protein to yield two separate circular monomers. Two families of recombinases are represented in various plasmid species: active-site tyrosine recombinases and active-site serine recombinases (62).

The ParA/*res* system of the broad-host-range, multidrug-resistant plasmid RK2, also known as RP4, is an example of a resolution system of the serine class. The ParA recombinase is produced from a three-open-reading-frame operon and acts at the linked recombination site *res*. RK2 *res* has an organization similar to that of the Tn*3* family of transposons, with three inverted repeats being bound by recombinase (16, 17). It contributes greatly to plasmid stability. The virulence plasmid of *Yersinia pestis*, pMT1, encodes a comparable resolvase (45).

Well-characterized tyrosine recombinase systems include the Cre/*loxP* recombination system of the P1 plasmid prophage (Fig. 1A) (3) and the ResD/*rfs* resolution system of the F plasmid of *Escherichia coli* (41). These systems contribute significantly to the stability of the plasmids and have been proven to act by resolution of plasmid dimers that accumulate due to recombination between plasmid sisters. The ResD/*rfs* resolution system is encoded within the RepFIA replication region of the F plasmid. *resD* is cotranscribed with the *ccdA* and *ccdB* genes, which are responsible for postsegregational killing. The target of ResD, *rfs*, lies upstream of this gene cluster (14, 41). An example of a related system in a virulence plasmid is the Rsd/*crs* system of the virulence plasmid pSDL2 of *Salmonella enterica* serovar Dublin (39). The *cis*-acting resolution site *crs* is located upstream of the resolvase gene *rsd* and contains eight direct incomplete 17-bp repeats followed by a segment of indirect repeats. This system contributes directly to plasmid stability (39). The *E. coli* virulence plasmid pB171 encodes Rsv, which is homologous to the ResD recombinase of the F plasmid (66).

Similar site-specific recombination systems are utilized by transposable elements for integration and excision from the genome and from other mobile elements. It is likely that dimer resolvases are derived from transposition resolvases, as is the case with ParA/*res* of RK2, which is derived from the Tn*3* family of transposon resolvases (17). The multimer resolution system of the multidrug-resistant plasmid pJHCMW1 appears to have been acquired from the transposon Tn*1331* (67).

Large plasmids are targets for transposition and often contain multiple transposons. Thus, in searching plasmid sequences for resolution systems, it is often unclear whether candidate sequences are involved in multimer resolution or in transposition. It seems likely that some loci serve both purposes. Also, recombination loci are likely to be involved in interplasmid recombination events that reassort gene cassettes between heterologous plasmids. Thus, site-specific recombination systems can be viewed as having multiple contributions to virulence. They stabilize virulence plasmids by resolving dimers and contribute to the plasticity of the available virulence functions by facilitating the lateral transfer of virulence cassettes and their reassortment into novel combinations.

POSTSEGREGATIONAL KILLING SYSTEMS

Postsegregational killing (PSK) systems eliminate plasmid-free cells that arise due to missegregation or replication errors (73). Such systems have also been termed toxin/antitoxin or plasmid addiction systems, and their activities have been described as programmed cell death. These plasmid loci have a gene for a stable antibacterial toxin that either kills or stops cell growth when expressed and a short-lived product that acts as an antidote to the toxin. When the plasmid is present in the cell, the antitoxin inactivates the toxin, but when the plasmid is lost, the antitoxin is degraded rapidly and the toxin kills the cell (33). In addition to these systems, termed type II PSK systems, there is a second class, type I, that acts in a similar way. Here

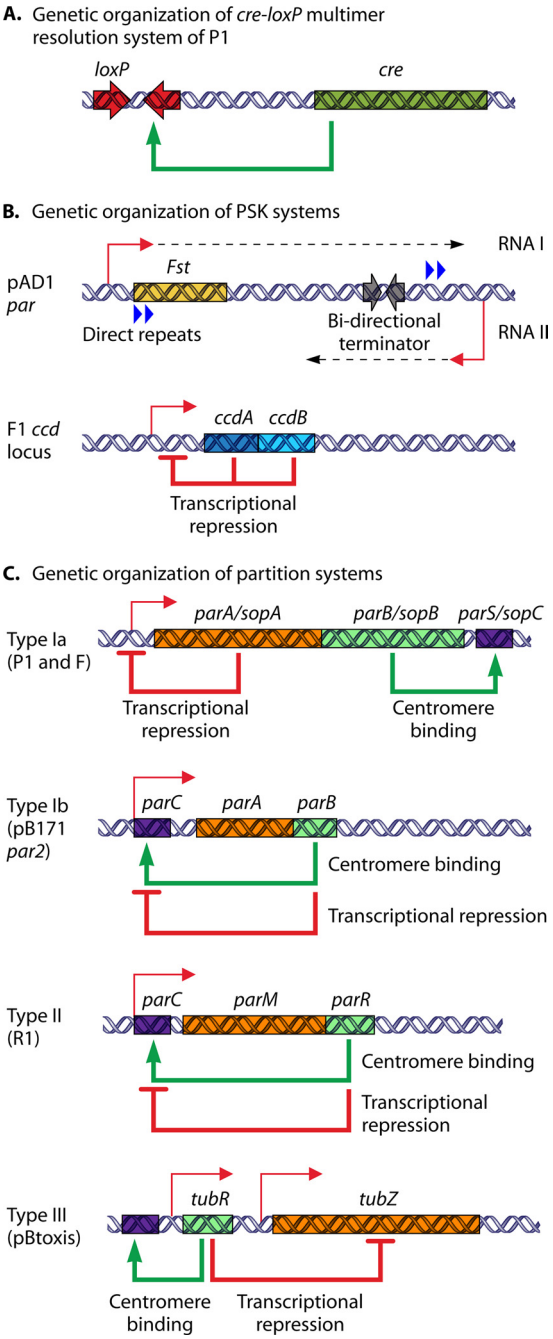

FIG. 1. (A) The Cre/*loxP* multimer resolution system of P1 has a *loxP* site 434 bp upstream of the *cre* gene (green box). It consists of two inversely oriented 13-bp recombinase binding motifs (red solid arrows) flanking an asymmetrical 8-bp sequence. (B) Genetic organization of PSK loci. The pAD1 *par* system consists of the *fst* gene (yellow box) encoding RNA I. RNA II is transcribed convergently and shares a bidirectional terminator sequence (gray solid arrows) and direct repeats at 3′ and 5′ ends (blue arrowheads). RNA II and I (dashed arrows) form a stable pair and inhibit *fts* translation. In plasmid-free cells, RNA II degrades rapidly, causing expression of the toxic Fst protein and resulting in cell death. The *ccd* system of the F plasmid consists of two genes, *ccdB*, encoding the killer, and *ccdA*, encoding the antidote. CcdA binds to CcdB and inhibits cell killing. In the case of plasmid loss, CcdA is rapidly degraded, causing CcdB-mediated cell death. The operon is autoregulated by the protein complex. (C) Genetic organization of the different types of partition systems found in plasmids. The green boxes and the orange boxes represent the centromere-binding protein and the motor protein, respectively. The centromere sequence has been marked by purple boxes. Red arrows mark the direction of transcription. Transcriptional repression and centromere binding have been marked in each case.

the "antitoxin" is an antisense RNA that blocks the function of the plasmid mRNA for a toxic protein. If the plasmid is lost, the antitoxin RNA degrades rapidly and translation of the more stable mRNA leads to toxin production and cell death (25, 29). Thus, only cells that successfully propagate the plasmid thrive in the population.

Plasmid R1 of *Escherichia coli* encodes the type I PSK system *hok*/*sok*. The Hok toxin associates with the cell membrane and acts by destroying the membrane potential of the cell, leading to a loss of energy for metabolism. The antidote, *sok*, is a small, unstable, *cis*-acting antisense RNA that inhibits *hok* translation (23). A similar system is found in the *Enterococcus faecalis* conjugative virulence plasmid pAD1. It is known as pAD1 *par* and encodes the mRNA, RNA I, for a 33-amino-acid toxic peptide, Fst, and an antisense RNA, RNA II (72). Fst, when translated, affects cell division and promotes membrane permeabilization, nucleoid condensation, and cell death (29). RNA I and RNA II are transcribed convergently and overlap at their 3′ bidirectional transcription termination sequences and 5′ direct repeat sequences. They form a highly stable complex via their 3′ and 5′ overlapping sequences and stop translation of *fts* (Fig. 1B) (71). RNA II is relatively unstable and is cleared from cells that lose the plasmid. The accumulated RNA I messenger is then free to produce the Fts toxin that kills the cells.

Well-characterized type II members include the *ccd* system of the F plasmid (52) and *phd*/*doc* of the P1 plasmid prophage of *E. coli* (43). In these systems, both the toxin and antitoxin are small proteins that are produced from adjacent genes on the plasmid. The antitoxin is unstable since it is particularly susceptible to degradation by host proteases. The F plasmid *ccdA* and *ccdB* genes form an operon that is autoregulated by its products (Fig. 1B). CcdB is a stable DNA gyrase inhibitor (10). When the plasmid is present, CcdA inhibits the interaction between DNA gyrase and CcdB. In cells that lose the F plasmid, the host Lon protease rapidly degrades the CcdA pool. In the absence of CcdA, CcdB binds to DNA gyrase and inactivates it, leading to DNA damage and cell death (33). The virulence plasmid MYS6000 of *Shigella flexneri* has a type II PSK locus, *mvpA*/*mvpT*, that has been shown to be important for plasmid maintenance (58). Type II PSK systems are characterized by two adjacent short open reading frames for the small toxin and antitoxin peptides. They have been found in large plasmids when functional tests have been used to locate them. However, they are a very diverse group and are not easy to recognize by DNA sequence alone.

Many plasmids carry specific restriction-modification (RM) loci. RM systems are composed of genes that encode a restriction enzyme that cleaves DNA in a sequence-specific manner and a modification methylase that modifies the target sequence so that the host genome is protected from cleavage (38). These systems can act as postsegregational killing systems. They help

in plasmid maintenance by killing plasmid-free cells because, on plasmid loss, the modification activity declines before the restriction activity, and the cells are killed by digestion of their DNA (38, 50).

PSK cassettes are often found in the host chromosome. Well-characterized chromosomal PSK systems of *E. coli* include MazE/MazF (20) and RelB/RelE (24). They are activated in response to elevated levels of ppGpp, which occur as a result of amino acid starvation. These systems are probably involved in stress response.

It is possible that chromosomal PSK systems are purely selfish DNA elements that ensure their own survival by killing host cells that lose them. However, they may also serve to preserve loci to which they are linked. PSK elements linked to large islands of virulence genes in the chromosome of a pathogen might prevent the loss of the islands as the pathogen population grows (57, 68).

PARTITION (*par*) SYSTEMS

Large plasmids have low copy numbers. There are sometimes only two or three copies in the dividing cell. Random distribution of these copies to daughter cells would lead to very high rates of plasmid loss (51). Under these circumstances, PSK systems would kill a large proportion of the cells in the population to the detriment of pathogen viability. Plasmids overcome this by having plasmid partition systems that ensure that each daughter cell receives at least one copy of the plasmid DNA (30). Partition systems direct the active segregation of the plasmids to either side of the cell center prior to cell division, minimizing the chances of plasmid loss. All low-copy-number plasmids appear to encode a partition (*par*) system. They consist of an ATPase or a GTPase motor protein, a specific DNA binding adaptor protein, and a *cis*-acting centromere-like site at which they act. The proteins are generally produced from an operon that is tightly autoregulated by one of the products (28).

Plasmid *par* systems can be classified into three types. Type I *par* systems encode a deviant Walker-type P-loop ATPase. Type II encodes an actin/hsp70 type of ATPase, and the recently described type III *par* system encodes a GTPase. Type I partition systems can be further classified into Ia and Ib (28). Type Ia systems have large ATPase motor proteins that serve as the operon autorepressor. Type Ib systems have smaller motor proteins and use the DNA binding protein as an autorepressor.

TYPE IA PARTITION SYSTEMS

The well-studied type Ia partition system is represented by the *par* system of the P1 plasmid of *E. coli* (44). It consists of a two-gene operon encoding the ParA ATPase, followed by a gene for the DNA binding adaptor protein, ParB (Fig. 1C). The operon is autoregulated by binding of ParA to its own promoter. Repression is further aided by binding of ParB to ParA (13, 34). The centromere-like *parS* site is located immediately downstream of the *par* operon. It consists of two types of repeat motif for recognition of ParB (64) and an integration host factor (IHF) site that binds the *E. coli* integration host factor (6, 12). Binding of IHF induces a bend in the DNA that facilitates ParB binding. Further copies of the ParB protein can load at the *parS* site, spreading out onto the surrounding DNA sequences (6, 56). *In vivo* studies of fluorescently labeled P1 plasmids show that they move apart to become roughly evenly distributed throughout the length of the cell. This process is aided by the pairing and active separation of copies that lie close to each other (59). The properties of the ParA protein suggest that ParA forms dynamic gradients in the cell that direct plasmid movement. This involves the complex interplay between the affinity of ATP-bound ParA for nonspecific host DNA and the ability of *parS*-bound ParB to stimulate ATPase activity of ParA, thereby releasing it from the host DNA (69).

The F plasmid of *E. coli* has a type Ia partition system, *sop* (Fig. 1C). This is organized similarly to P1 *par*, but the partition site (*sopC*) consists of 12 48-bp repeats and has no IHF site (49). Type Ia partition systems are widely distributed among virulence plasmids in Gram-negative pathogens. Those closely related to P1 *par* are particularly prevalent. Examples include pMT1 *par* of *Yersinia pestis* (74) and pWR100 *par* of *Shigella flexneri* (60).

TYPE IB PARTITION SYSTEMS

The type Ib systems have a small ParB DNA binding protein and a DNA organization different from that of type Ia. The type species of such loci is the *par2* locus of the *E. coli* virulence plasmid pB171 (Table 1) (19). The region upstream of the operon contains both the partition site *parC* and the operon control locus at which ParB acts as an autoregulator (Fig. 1C). ParA nucleates onto the ParB/*parC* DNA complex, forming filamentous polymers. When two plasmids are close to each other, the complexes interact, causing disassembly of the ParA filaments that lie between them. This causes the plasmids to move outward, following the retreating edge of the ParA filament field. The result is a roughly equal distribution of the plasmids along the long axis of the cell (55). The principal difference between the latest models for type Ia and type Ib plasmid movement by ParA action lie in the proposal that ParA acts as a dynamic concentration field in the former (69) and as filaments in the latter (55). The models otherwise have key similarities, and it seems likely that the mechanisms differ in detail but are otherwise basically the same (36).

The pAD1 plasmid of *Enterococcus faecalis* is a virulence plasmid that has a type Ib partition system. pAD1 is a low-copy-number, pheromone-responsive plasmid that produces a cytolytic exotoxin that enhances virulence (9). The partition protein RepB, an ATPase of the ParA family, is followed by RepC. The partition site lies upstream of *repB* and contains a group of 25 8-bp direct repeats (21). RepB (33 kDa) and RepC (14.4 kDa) were both shown to be required for maximal stabilization. RepC binds to the iteron region, and RepB can then interact by a RepB/RepC contact. Although this system shows some differences in organization from that of pB171 of *E. coli*, the sequence similarity and indications of a common mechanism show that the type I partition family spans both Gram-positive and Gram-negative pathogens. Similar systems are to be found in several other Gram-positive species, suggesting that the type Ib systems are broadly distributed (28, 32).

TYPE II PLASMID PARTITION SYSTEMS

Type II *par* systems contain a member of the actin/hsp70 superfamily of ATPases (5), as exemplified by the *par* locus of antibiotic resistance plasmid R1. The *parMRC* locus of R1 encodes an actin homologue, ParM, and a DNA binding adaptor protein, ParR, and contains an upstream centromere-like region, *parC* (Fig. 1C) (26, 27). The promoter of the operon lies within *parC* and is autoregulated by cooperative binding of ParR (11, 37). ParM, in the presence of ParR and *parC*, forms dynamic actin-like filaments (22, 47, 48). ParM filaments grow by insertion of ParM-ATP molecules at the ParR/*parC* site. The ParR/*parC* complex stabilizes the ParM filament (22). *In vivo* studies have shown that ParM filaments force plasmid pairs rapidly to opposite cell poles. After reaching the poles, the ParM filaments rapidly depolymerize, and the segregated plasmids resume random diffusive motion. Plasmids can undergo several rounds of segregation in a single cell cycle (7).

This type II mechanism clearly differs from that of the type I systems. Rather than involving disassociation of a ParA structure from the region between plasmids, the ParA structure grows between them and forces them apart. The final distribution of copies is not even along the cell length. Rather, most copies end up at the cell poles. This is because the rigid filaments often continue to push the copies apart until they reach the ends of the cells (47).

Type II systems appear to be less prevalent in nature than type I systems. Interestingly, the *E. coli* virulence plasmid pB171 contains both a type II and a type Ib partition system, and both have proven to be active in partition (19).

TYPE III PARTITION SYSTEMS

The large virulence plasmid pXO1 of *Bacillus anthracis* encodes a RepX protein that was originally thought to be part of the replication machinery. RepX is distantly related to the bacterial cell division protein FtsZ. It is a GTPase and has a motif that is common to tubulins (65). RepX forms GTP-dependent filamentous structures both *in vitro* and *in vivo* and shows nonspecific DNA binding. It is likely that it is involved in plasmid partition (1, 2). The pBtoxis plasmid of *Bacillus thuringiensis* encodes a RepX homolog, TubZ, that is required for plasmid stability. It assembles into dynamic filaments *in vivo* (42). An upstream DNA binding protein, TubR, regulates TubZ expression. TubR binds to a *cis*-acting sequence of four repeats that lie upstream of the genes for the protein components (Fig. 1C). TubZ forms dynamic filaments that elongate at one end and retract at the other. By associating with these filaments, the plasmid/TubR complex is presumed to move with the progressing filament to achieve segregation (42). In this novel class of elements, termed type III, the GTPase (RepX, TubZ) appears to play the role of a ParA analog and TubR the role of ParB.

PARTITION OF LINEAR PLASMIDS

Very little is known about the partition system of linear virulence plasmids. Bacteriophage N15 of *E. coli* is maintained as a linear plasmid molecule with covalently closed ends. It is stably maintained at 3 to 5 copies per bacterial chromosome. Stable inheritance is ensured by a plasmid-carried *sop* locus that is homologous to F *sop*. The locus consists of a two-gene operon, *sopA* and *sopB*, which are similar to F *sop*. But unlike the case of the F plasmid, the centromere sequence of N15 consists of four individual inverted repeats scattered over the 12-kb N15 genome (54). Stabilization of the linear plasmid depends on the number and position of the centromere site (15). Similar partition systems have been identified in other linear plasmids, such as pY54 of *Yersinia enterocolitica* (35) and φKO2 in *Klebsiella oxytoca* (8). Unlike circular plasmids, scattered centromere-like sites appear to be a prerequisite for stable inheritance of linear plasmids.

HOW PLASMID MAINTENANCE FUNCTIONS COMBINE TO PRODUCE HIGH LEVELS OF PLASMID STABILITY

Cells containing low-copy-number plasmids often encounter environments where the resident plasmid is dispensable for growth. Thus, the plasmid must be stably maintained to prevent its loss from the population. In practice, naturally occurring plasmids are highly stable, with loss rates that are hardly measurable over many generations. Plasmid replication and maintenance systems combine to achieve this (Fig. 2). Replication doubles the number of plasmids in each generation. Some of these copies form dimers by recombining with each other. These dimers are efficiently reduced to monomers by the multimer resolution system. Plasmid monomers are actively partitioned to opposing cell halves by the action of the partition system. Cell division then produces new cells, each of which contains a plasmid. This basic plasmid cell cycle is prone to occasional errors that lead to the production of a few plasmid-free cells (Fig. 2). These cells are subject to postsegregational killing that removes them from the population. All three of the plasmid maintenance systems are essential for physiological levels of segregational stability (51) (Fig. 2). Without multimer resolution, insufficient plasmid molecules will be present in many cells for proper segregation. Without active plasmid partition, too many cured cells will be produced. Killing many cells would severely compromise the viability of the population. Without postsegregational killing, the inevitable mistakes made by the replication and partition and multimer systems of the plasmid will allow the gradual accumulation of plasmid-free cells, which will eventually take over the population. Thus, all three types of system are important for physiological stability, and all three are likely to be found on each naturally occurring plasmid type (51).

In practice, plasmids like P1 that have the very low copy numbers illustrated in Fig. 2 have error rates for replication, multimer resolution, and partition that combine to produce about one plasmid-free cell per thousand new-born cells per generation under laboratory conditions. Postsegregational killing of the plasmid-free cells reduces this to less than 1 cell in 10^8 (58). Most large plasmids are likely to have somewhat higher copy numbers. In these cases, the rate of loss will be further reduced due to the fact that extra copies reduce the probability that replication or segregation errors will give rise to plasmid-free cells.

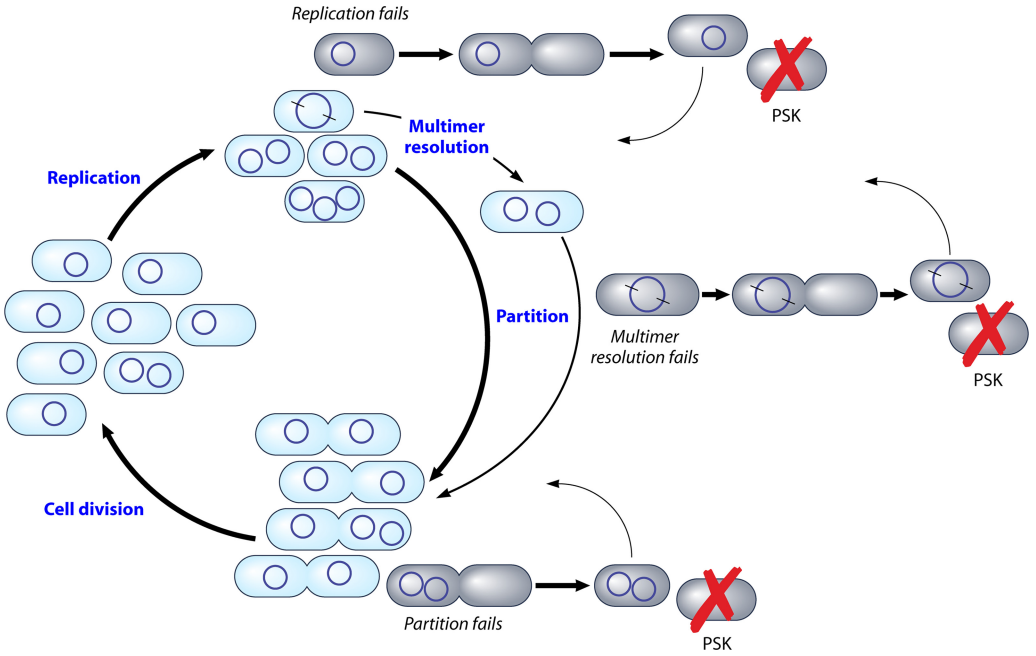

FIG. 2. How plasmid maintenance systems combine to achieve stable plasmid inheritance. The cell cycle of typical cells (blue cells) containing a low-copy-number plasmid is represented on the left. Plasmid replication doubles the number of plasmids in each cell. Some cells contain plasmid dimers formed by generalized recombination. These are reduced to monomers by the multimer resolution system. The replicated plasmids are subjected to active partition to opposite cell halves, ensuring that cell division produces two cells, each of which contains at least one plasmid copy. The gray cells represent those rare cells where the replication, multimer resolution, or partition system of the plasmid has failed to function properly. In each case, cell division produces one plasmid-containing cell that is returned to the general population and one that has no plasmid copy. Postsegregation killing is triggered in the latter cells, killing them and thus ensuring that all viable cells in the population retain the plasmid.

THE CASE FOR UBIQUITY OF THE THREE TYPES OF PLASMID MAINTENANCE FUNCTION

We have argued that all three types of plasmid maintenance system are essential for the stable inheritance of large plasmids in the absence of strong selections for their presence. Most pathogenic bacteria have opportunities to grow in the environment outside the host, in which virulence functions give them a selective advantage, so that this rule should apply to the virulence plasmids that they carry. Since the genomes of virulence plasmids are large and complex, exhaustive analysis of gene function is usually lacking. The most complete information available is for the virulence plasmids of Gram-negative enteropathogens (Table 1). Here, all three types of system are present. In addition, sporadic information from other classes suggests that at least one member of all three of these classes is present on any given virulence plasmid. Indeed, virulence plasmids often show evidence of more than one copy of each type. For example, the annotated sequence of the large virulence plasmid of *Shigella flexneri*, pWR501 (70) (Table 1), indicates the presence of two plasmid partition systems: homologues of the P1 *parA*/*parB* (type Ia) and R1 *stbA*/*stbB* (type II) *par* systems, respectively. It contains at least two toxin/antitoxin systems, one related to F *ccdA*/*ccdB* and another to pMYS6000 *mvpA*/*mvpT*. It also has a homologue of RelB, a toxin encoded by the *relB*/*relE* PSK locus (31). Plasmid pWR501 also carries a sequence similar to that of the ColE1 *cer* site (61) that is involved in plasmid multimer resolution, and there are several putative transposases and resolvases included in the annotated sequence. Cursory examination of the sequences of many other virulence plasmids suggests that similar patterns are frequent. Naturally, it cannot be discounted that unknown types of plasmid maintenance systems are yet to be discovered, but the obvious problems that threaten plasmid maintenance appear to be handled very efficiently by the combination of the three system types described here.

WHY ARE MULTIPLE SYSTEMS OFTEN PRESENT?

In practice, the plasmid maintenance functions of a given virulence plasmid are not limited to one of each class. There is no obvious reason why two or more dimer resolution systems should interfere with each other, and the error frequency of resolution should be reduced if more than one is present on a given plasmid. The mechanisms involved in plasmid partition are also tolerant of having more than one partition system on the same plasmid (4). Presumably, having two such systems can also improve fidelity by minimizing failures. Finally, multiple postsegregational killing systems are often found on a single plasmid. Again the fidelity of plasmid maintenance should be increased because the efficiency of killing of any plasmid-free cells should be increased. Loss of the plasmid should trigger the action of the toxin associated with each PSK system present, giving an additive effect for killing efficiency. Thus, the presence of multiple elements of different specificities for each of the three major classes of plasmid maintenance element is not unexpected and is likely to be beneficial for the overall fidelity of plasmid maintenance.

THE PRACTICAL CONSEQUENCES

We propose that large, low-copy-number virulence plasmids encode at least one copy of all three of the major types of plasmid maintenance system. This is likely to apply to all such plasmids that are circular and are replicated in the usual theta mode. Since virulence plasmids encode virulence functions and maintenance of the plasmid is important for virulence, the three types of plasmid maintenance system can themselves be regarded as ubiquitous virulence factors. When analyzing new virulence plasmids, the presence of such systems can be anticipated. Moreover, their presence and properties could have consequences in searches for novel therapies for infectious disease.

Plasmid partition systems are unique to bacterial plasmids and to the chromosomes of some bacterial species. Although targeting these is a rather indirect way to prevent infection, it is nevertheless possible that drugs designed to target them will prove useful, and some efforts along these lines are under way.

Postsegregational killing systems offer a different potential for therapy. Each of these systems produces a small peptide toxin capable of targeting some vital target of the host. First, although peptides do not in general make good drugs, it is conceivable that drugs could be modeled on them. Second, these killer systems can be regarded as very successful selfish elements that rely on targeting of host functions in order to survive. Thus, it can be argued that the host targets are vital and are not easily mutated to resist the toxin. Such targets could prove to be ideal for the design of novel antibacterial drugs.

Knowledge of plasmid maintenance systems is also important for the design of stable plasmid vectors used for medical purposes. Attenuated *Shigella* and *Salmonella* strains have been used as carriers for delivering foreign vaccine antigens to mammalian cells. One of the major problems associated with the use of live bacterial carriers is the instability of the plasmids used to encode the antigen. Knowledge of the functional interplay of plasmid maintenance functions should prove useful here, and incorporation of PSK and partition functions into the vectors has already given positive results (46). Vaccine antigens, naked DNA antigens, and therapeutic agents are increasingly made from plasmids in producer bacteria in liquid culture. The maintenance stability of the plasmid vectors can be a frequent problem, especially in continuous-flow culture (40, 53). Here again, detailed knowledge of the interplay of the three maintenance system types should prove useful in vector design.

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